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Influence of temperature on yolk utilization by the white sturgeon, *Acipenser transmontanus*

Y. L. WANG*, R. K. BUDDINGTON†‡ AND S. I. DOROSHOV*

*Department of Animal Science, University of California, Davis, California 95616, U.S.A.
†Department of Physiology, UCLA Medical Center, Los Angeles, California 90024, U.S.A.

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The utilization of yolk nutrients by the white sturgeon was investigated at incubation temperatures of 11, 14, 17, and 20° C. The rates of development and dry matter loss are temperature-dependent, with an upper critical temperature between 17 and 20° C above which survival is reduced (the temperature range investigated was not sufficient to allow determination of a lower critical temperature). The patterns of yolk utilization are similar to those of other fish. Protein depletion occurs at relatively constant rates throughout yolk nutrition, with the highest rate observed at 20° C. More than 95% of the original lipid levels were still present at hatch, except at 20° C (88%). After hatch, lipid content rapidly decreased at all temperatures, with fish incubated at 11° C possessing higher lipid levels at yolk depletion. The question of why the temperature tolerances of early life stages are more limited than those of older fish is discussed.

I. INTRODUCTION

The majority of aquatic environments are usually neither constant nor predictable. As a result the eggs of fish may be exposed to a variety of conditions during the incubation period. Selection has favoured those individuals whose eggs can develop within the range of anticipated environmental conditions. Since temperature is generally the most variable environmental parameter and also the most controllable hatchery condition, it has been the most thoroughly investigated environmental factor influencing fish development.

The Salmonidae have received most of the research effort and our current knowledge of metabolic aspects of embryonic and larval fish development is primarily based on studies of eggs which are relatively large and are adapted for low temperatures and long incubation periods. For comparative purposes there is a need to include a greater diversity of species with different reproductive strategies. The limited information now available does, however, reveal the existence of species variation in the patterns and rates of yolk utilization as well as the growth of embryos and larvae in response to different incubation temperatures (reviewed by Heming & Buddington, in press). Data from the various studies demonstrate species-specific ranges of incubation temperatures within which yolk utilization efficiency and survival are maximized.

Development rates and survival of chondrosteans are affected by temperature (Detlaf *et al.*, 1981; Wang *et al.*, 1985). For example, Wang (1984) observed that size of white sturgeon embryos at hatch is inversely related to incubation temperature. His observations and measurements of yolk sac size relative to body length indicate that higher temperatures result in the embryos hatching at an earlier

‡To whom reprint requests and correspondence should be addressed (Tel: 213-825-6076).

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development stage. Chondrosteans are somewhat unique among fish in that they develop holoblastically and possess an intraembryonic yolk endoderm (Detlaf & Ginzburg, 1954); because of this, the yolk sac participates in formation of the gut, and yolk materials are present within the presumptive stomach and intestine. In contrast in fish that develop meroblastically, characteristic of most teleosts, the yolk sac is an extraembryonic structure. Presently, there is only a limited amount of information concerning the utilization of yolk materials by embryos which develop holoblastically, such as the chondrosteans, and virtually nothing is known concerning the influence of temperature. We, therefore, investigated the rates of development and utilization of yolk by embryos and larvae of white sturgeon which were incubated at different temperatures.

II. MATERIALS AND METHODS

PROCUREMENT AND INCUBATION OF EGGS AND LARVAE

Gametes (eggs and sperm) were obtained from one feral and several male white sturgeon and fertilization followed Doroshov *et al.* (1983). Two hundred millilitres of fertilized eggs were stocked into 2-l incubation jars with a bottom flow inlet (Wang *et al.*, 1985). Sixteen stocked jars were positioned in a corresponding number of 15-l rearing tanks, each provided with aeration. Groups of four tanks with incubation jars were incorporated into isolated, semi-closed recirculation systems which were maintained at 11, 14, 17, and 20° C. Each system was provided with constant water inflow such that the entire water volume was turned over twice each day. Following hatch, the fry spilled out of the incubation jars and were collected and held in the rearing containers.

Following Balon (1975) we use here the term 'embryo' for developing fish prior to hatch, and 'eleutheroembryo' for post-hatch, non-feeding fish that are still dependent on yolk reserves.

SAMPLING AND PREPARATION OF MATERIALS

Samples for analysis were removed at the stages of neurulation (stage 22), hatch (stage 36), appearance of pyloric sphincter (stage 40), and yolk depletion (stage 44) as described by Detlaf *et al.* (1981) and Wang *et al.* (1985). The times required to reach each developmental stage at the different incubation temperatures are presented in Fig. 1. To provide adequate quantities of material for proximate analysis, we pooled approximately 600–1200 individuals from the four jars at each temperature. Because of lower survival we did not sample the 20° C treatments at stage 40. The developing fish were homogenized with approximately equal volumes of chilled distilled water and were then lyophilized. The resulting material was stored at −20° C until analyzed. Because of the intraembryonic nature of the yolk sac, we did not attempt to isolate the yolk materials from the somatic tissues of the embryos and larvae; nor were the egg membranes removed from the embryos. Therefore, proximate composition values reflect the developing fish plus the associated yolk and, prior to hatch, also the egg membranes and perivitelline fluids.

PROXIMATE ANALYSES

Dry matter determinations were performed on 10 individual embryos (with egg membranes and perivitelline fluids prior to hatch) or eleutheroembryos by dessication at 65–70° C (for 96 h). At hatch we also recorded the wet and dry weights of 10 larvae with intact egg membranes. This allowed us to quantify the wet weight and dry matter associated with the egg membranes and perivitelline fluids by comparison with recently hatched larvae. A mean and S.E.M. of dry matter content per individual was calculated for each treatment.

Caloric content of the lyophilized material was ascertained by microbomb calorimetry of 0·5-g samples. Protein content was measured by the method of Lowry *et al.* (1951). Lipids were extracted from the lyophylizate by a 24-h refluxing soxhlet extraction with petroleum

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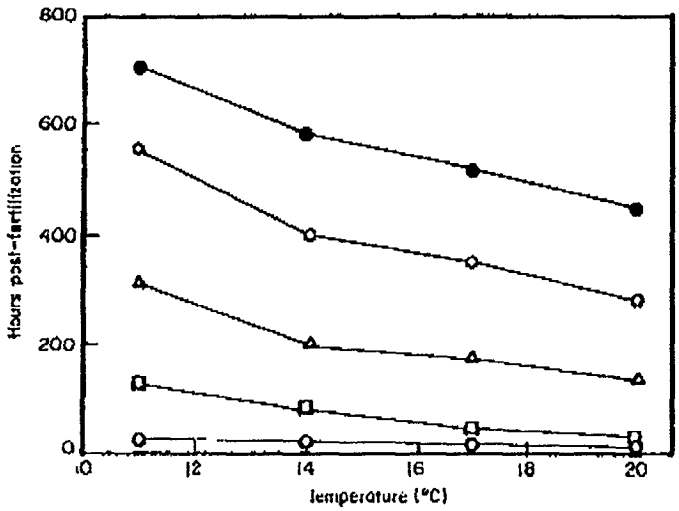

FIG. 1. Hours post-fertilization required to attain specific developmental stages when white sturgeon eggs and fry are incubated at 11, 14, 17, and 20° C. Key: ○, 3rd cleavage; □, closure of neural tube; △, complete hatch; ◇, formation of pyloric sphincter; ●, yolk depletion.

ether. The petroleum ether was then evaporated (30–35° C) and the resultant lipid weighed. Ash content was measured following ignition of the samples (550° C, 24 h). Results of the analyses were expressed as percentages of original yolk quantities, mg mg^{-1} dry matter, and mg per individual developing fish on a wet and dry basis.

III. RESULTS

DEVELOPMENT RATE AND WET AND DRY WEIGHTS

The rate of development was temperature-dependent (Fig. 1). Relative to 11° C, the time required to attain specific developmental stages decreased, on average, by 25% at 14°, 48% at 17°, and 58% at 20°.

An increase of incubation temperature resulted in smaller eleutheroembryos at hatch and at yolk depletion: there were significant decreases ($P<0{\cdot}05$) in body length at hatch at higher incubation temperatures (Table I). The decline of dry matter during development was also accelerated at elevated temperatures. The dry matter associated with the egg membranes and perivitelline fluid was similar for all treatments and averaged $0{\cdot}97 \pm 0{\cdot}03$ mg. Hence, the percentages of dry matter present in the unfertilized egg (100%) which was retained at hatch were 99, 98, 93, and 82% at the respective experimental temperatures of 11, 14, 17, and 20° C. Corresponding values at yolk depletion were 62, 59, 55, and 48%. These results indicate that (1) the rate of dry matter loss is directly related to incubation temperature, and (2) the decrease of dry matter is accelerated after hatch, corresponding with greater activity of the developing fish.

In contrast to dry matter, wet weight increased during embryonic development at all experimental temperatures (except when the chorion was lost at hatch); this was caused by an increase of moisture content. Although not significant ($P>0{\cdot}05$), embryonic wet weights tended to be lower at elevated temperatures.

TABLE I. Weights (mg) and lengths (mm) of developing white sturgeon at the different stages when incubated at 11, 14, 17, and 20° C. Values are means ± S.E.M. for an individual larva. W = wet weight, D = dry weight, L = length (from tip of snout to tip of tail). Values with different superscript letters indicate significant difference ($P < 0.05$) between different temperature treatments

Temperature	Parameter	Developmental stages				
		0*	22	36	40	44
11	W	26·3 ± 0·1	30·0 ± 0·1^a	22·0 ± 0·1^a	27·2 ± 0·1^a	30·8 ± 0·3^a
	D	8·25 ± 0·02	8·33 ± 0·05^a	7·83 ± 0·06^a	5·98 ± 0·06^a	4·65 ± 0·06^a
	L			13·0 ± 0·1^a		
14	W		28·0 ± 0·2^b	19·9 ± 0·1^b	29·0 ± 0·1^b	36·9 ± 0·3^b
	D		8·45 ± 0·03^b	7·35 ± 0·06^b	5·85 ± 0·05^a	4·40 ± 0·08^b
	L			12·7 ± 0·1^b		
17	W		26·6 ± 0·4^c	17·0 ± 0·4^c	33·9 ± 0·4^c	35·7 ± 0·5^b
	D		8·58 ± 0·06^b	7·03 ± 0·09^c	6·48 ± 0·08^b	4·15 ± 0·06^c
	L			12·3 ± 0·1^c		
20	W		28·2 ± 0·3^b	16·0 ± 0·3^d	26·2 ± 0·3^a	26·8 ± 0·6^c
	D		8·67 ± 0·02^c	6·18 ± 0·30^d	6·18 ± 0·24^a	3·64 ± 0·21^d
	L			11·2 ± 0·2^d		

*Pooled data for eggs at fertilization.

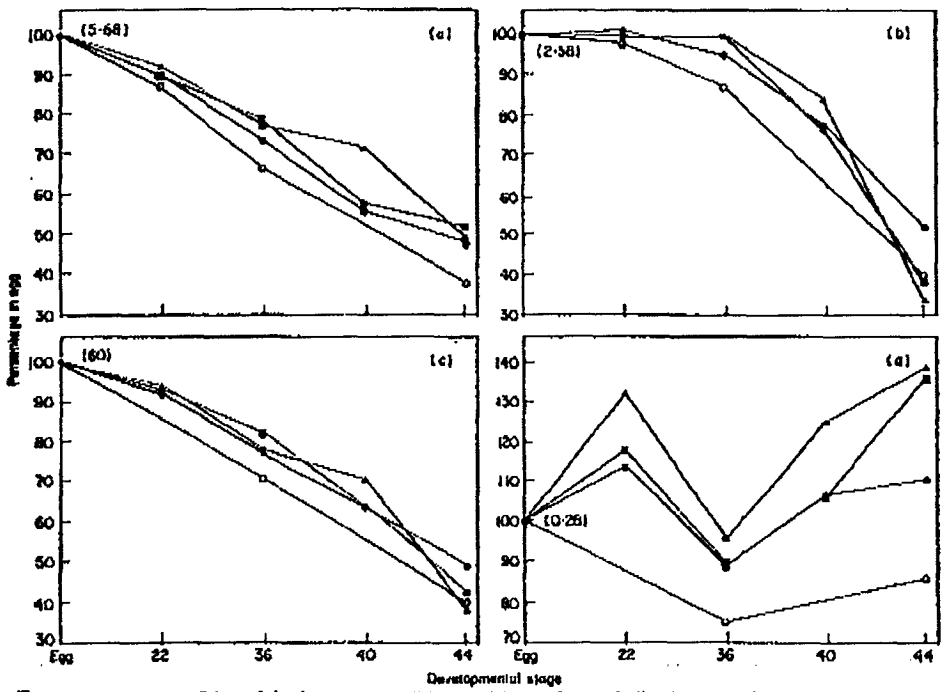

FIG. 2. Percentages of the original (a) protein, (b) lipid, (c) calories, and (d) ash present in the unfertilized egg remaining in the developing life stages of white sturgeon incubated at 11, 14, 17, and 20° C. Values in parentheses represent the original mg per individual egg and calories per individual egg. Key: ●, 11°; ■, 14°; ▲, 17°; ○, 20°.

After hatch, wet weights increased but not in a direct relationship with temperature. Eleutheroembryos attained the greatest wet weights at 14 and 17° C, with those reared at 11 and 20° C being lighter. Whereas the lower wet weights at 20° C were partially caused by a lower dry matter content, the low value at 11° C was due to a reduced moisture content.

BIOCHEMICAL CHANGES

The biochemical data (Fig. 2) reveal changes in proximate composition during the non-feeding development of white sturgeon reared at the experimental temperatures.

Protein, the primary constituent of the dry matter of the fertilized ova (5·68 mg; 67% of the dry weight), exhibited a consistent decrease throughout development in all treatments [Fig. 2(a)]. Protein contents of sturgeon incubated at 20° C, however, were consistently lower than those at other temperatures, which were similar. As a result, at yolk depletion the percentage of yolk protein converted into eleutheroembryo ranged from 48 to 52% for sturgeon reared at temperatures from 11 to 17° C but was only 38% for those at 20° C.

Most of the fat content of the ova (2·58 mg; 30% of the dry weight) was retained during embryonic development [Fig. 2(b)]. Only at 20° C did fat content drop below 90% of the original quantity by the time of hatch. Following hatch, there was a rapid decline in fat within all treatments. When the yolk was exhausted

34–40% of the original fat had been retained in larvae at 14–20° C. Fish at 11° C retained 52% of the initial lipid provided in the ova.

Changes in caloric content [Fig. 2(c)] corresponded to changes in protein and fat percentages. For example, during the egg phase the decrease of calories can be attributed primarily to the utilization of protein. During this period there was comparatively low utilization of the higher caloric density lipids. Because of the retention of lipid, the rate of decrease of protein during the egg phase was slightly greater than that for the caloric content. The increased utilization of lipid after hatch resulted in slightly lower percentage of original egg calories remaining at yolk depletion, relative to protein. However, the observed differences between the rates of depletion of the original protein content and caloric density due to shifts in the utilization of lipid were not significant. This was due to the presence of over twice as much protein as lipid in the egg (5·68 mg v. 2·58) which would reduce the influences caused by changes in lipid content. Although fish reared at 20° C had a lower caloric density at hatch, when the yolk was depleted fish reared at 14–20° C were similar in caloric content. In agreement with lipid data, an incubation temperature of 11° C resulted in a higher caloric density when the yolk was depleted.

The large increase of ash content after fertilization [Fig. 2(d)] was due to the hatchery procedure of silt-treating the eggs which results in the adhering of silt to the egg membrane. This is performed to eliminate the adhesiveness of the eggs and allow them to be incubated in the hatching jars without clumping. When the egg cases were shed at hatch, the ash content decreased to levels which were slightly lower than prior to the siltation procedure. Again, values from fish reared at 11–17° C were similar whereas those from 20° C had a lower ash content at hatch. When the yolk was depleted, fish reared at 14 and 17° C had higher ash levels than did fish incubated at 11° C, and especially at 20° C.

IV. DISCUSSION

The influence of temperature on the development rate of white sturgeon eggs was similar to that described for other acipenserids (Detlaf *et al.*, 1981; Nikol'skaya & Sytina, 1978; Detlaf & Ginzburg, 1954). The combined data from the various studies, including the present one, indicate that the developmental responses of the holoblastic eggs of chondrosteans to different temperatures are, in general, similar to those of meroblastic eggs. Hence, when the incubation temperature is increased there is an acceleration of development, which apparently causes a greater proportion of the yolk reserves to be channelled into catabolic rather than anabolic processes. As a result, size based on dry matter content of the embryos and eleutheroembryos is inversely related to temperature, as indicated in Table I.

The pattern of nutrient utilization in white sturgeon eggs is similar to that reported for eggs of other fish, regardless of cleavage type. A relatively constant decrease in protein content throughout pre-feeding development has been reported for salmonids (Heming, 1982) and other fish (reviewed by Heming & Buddington, in press). The trend of lipid conservation prior to hatch, followed by a decrease during the eleutheroembryo phase, is also consistent among fish.

Each species has a preferred temperature range for egg incubation within which egg survival is high and utilization of yolk nutrients is relatively efficient and

constant. The upper limit of the range for the white sturgeon investigated is somewhere between 17 and 20° C, with a minimum of 11° C or lower. Within this range, only development rate is significantly affected. The amounts of yolk nutrients utilized by the developing fish incubated at 11–17° C were not different. At hatch, eleutheroembryos from eggs incubated within this range were similar in caloric content and percent of fat and protein. In contrast, newly hatched fish at 20° C were lower in calories, fat and protein. During the eleutheroembryo phase the decrease in protein content was similar for larvae at 11–17° C. Hence, conversion of yolk proteins to tissues was not affected within this range of temperatures. The increased rate of protein decline in eggs incubated at 20° C indicates that the elevated temperature resulted in a greater utilization of the yolk proteins. Based on patterns of nitrogen excretion, Kaushik *et al.* (1982) also observed increased protein utilization by carp eggs incubated at higher temperatures. After hatch, the rate of depletion of yolk lipids is related to incubation temperatures: this is apparently associated with increased utilization of lipids to supply additional energy for the heightened activity (personal observations) and higher metabolic rates at the higher temperatures. Curiously, at 20° C, eleutheroembryos contained lipid levels at yolk depletion that were higher than anticipated: two possible explanations of this are (1) that at 20° C utilization of yolk lipids may be impaired, and (2) that we may have sampled the 20° C, yolk-depleted eleutheroembryos at a relatively earlier stage than those from the other temperature treatments, so they may not have utilized the lipids to a degree consistent with their higher metabolic rates at elevated temperature.

A review of the literature indicates that 14–16° C is the optimal temperature for incubation of eggs of the various acipenserids (Wang *et al.*, 1985; Detlaf *et al.*, 1981), which corresponds with our findings. It is surprising that there is little interspecies variation despite a diversity of natural temperature ranges. For example, Siberian sturgeon, *Acipenser baeri*, inhabit northern Siberian streams with water temperatures generally less than 10° C (Votinov & Kasjanov, 1974) whereas sevryuga, *A. stellatus*, and paddlefish, *Polyodon spathula*, occur in water at temperatures usually greater than 20° C. However, the optimal incubation temperature for eggs of all three species is approximately 14–16° C (Detlaf *et al.*, 1981; Ballard & Needham, 1964). The white sturgeon of the present study were from the stock in the Sacramento River, California and which spawn during the spring when water temperatures are 14–16° C (Kohlhorst, 1976): survival data (Wang, 1984), the present results, and studies of white sturgeon culture (Doroshov *et al.*, 1983) reveal that this is the optimal temperature range for eggs from fish of this population. Our data with those of Wang *et al.* (1985) indicate that temperatures above 17° C result in increased mortality, smaller fish at hatch, and lower efficiency of yolk utilization, and that there is virtually no post-hatch survival at temperatures greater than 20°. We were unable to establish a lower critical temperature, but minimum incubation temperatures reported for other chondrosteans range from 6 to 8° C (Detlaf *et al.*, 1981). If the chondrosteans share a common zone of thermal plasticity within which survival and yolk utilization efficiency are relatively constant, then a lower incubation temperature level of 6–8° can also be speculated for the white sturgeon.

When incubation temperatures are outside of the optimal range, development appears to be inhibited at early stages, generally near the time of gastrulation. This

results in either death of the embryo or the development of abnormalities. Further deviation from the optimal range causes increasing mortality. This pattern has been observed in white sturgeon (Wang *et al.*, 1985) as well as other species (Detlaf *et al.*, 1981).

Apparently, the early life stages of most fish are unable to compensate for temperature fluctuations. Hence, fish should be adapted to spawn within specific temperature ranges. The lowered success when incubation occurs outside of the range may be attributed to a variety of factors, all of which would ultimately impinge on cell functions. Corresponding with this, temperature is a major stimulus for onset of reproductive activities in various fish species (reviewed by Lam, 1979).

The reasons why temperatures outside of the optimum elicit death or development of abnormalities are not clear. For instance, why do the eggs of most acipenserids exhibit an increase of abnormal development or mortality when incubated at temperatures above 20° C or below 8° C? These temperatures would not cause protein denaturation and should not abolish enzyme activites. One potential, but as of yet unexplored, explanation may be compromised membrane functions. If early developmental stages are not able to alter the fatty acid composition of their cell membranes in response to different temperatures, then temperatures deviating from the optimum would result in changes of cell membrane fluidity and would compromise membrane functions such as ionic regulation. A review of the literature indicates that lipid biosynthetic capacities increase with age and that early developmental stages of fish may be unable to alter their membrane lipid composition (Terner *et al.*, 1968). Prior to hatch there is little variation in fatty acid composition (Nakayama & Tsuchiya, 1976) and the ratio of saturated to unsaturated fatty acids remains unchanged (Hayes *et al.*, 1973). Although none of these studies determined the influence of temperature on lipid composition of early stages, the existing data do suggest that fish embryos prior to gastrulation lack sufficient metabolic capabilities to synthesize fatty acids or alter those provided in the yolk. Therefore, it is unlikely that early embryos exposed to changing temperatures are able to adapt the composition of their cell membranes, hence fluidity of their cell membranes resulting in cell dysfunction. There is a need to explore further the metabolic capacities of developing fish and also investigate possible differences between stenothermal and eurythermal eggs. Another potential subject for research is how the quantity and fatty acid composition of lipids in the maternal diet may influence the lipid composition of the eggs; then, in turn, how the lipid composition of the eggs might influence the temperature range within which the eggs can survive and develop normally.

The similarity in survival and yolk utilization efficiency of white sturgeon eggs incubated at between 11 and 17° C could be exploited by hatchery operators. Higher incubation temperatures will result in an earlier hatch of somewhat smaller eleutheroembryos (Wang, 1984), but the smaller size is due primarily to the embryos hatching at an earlier developmental stage (Wang *et al.*, 1985). When feeding is initiated fish size is comparable, regardless of incubation temperature. This phenomenon has also been reported for other fish (Heming, 1982). A benefit of the higher temperatures is the shorter incubation period and earlier onset of exogenous feeding. Hence, the earlier feeding will allow a hatchery operator to realize the exponential growth associated with feeding at an earlier date. The

thermal plasticity exhibited by chondrosteans with respect to incubation temperatures will also obviate the requirement for precise temperature control.

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